

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A method for detecting translocation of a component fused to a luminophore extracting quantitative information relating to an influence on redistribution of at least one component in the a cell in mechanically intact or permeabilised living cells, the method comprising:

recording detecting translocation of variation in spatially distributed light emitted from a from said luminophore,
wherein

said translocation is detected by measuring changes in luminescence intensity,

said luminophore is encoded by and expressed from a nucleic acid sequence in said cell, and

said translocation is from cytoplasm to membrane, from membrane to cytoplasm, from an aggregated form to a dispersed form or from a dispersed form to an aggregated form

the luminophore being present in the cells and being capable of being redistributed in a manner which is related with the degree of the influence, and/or of being modulated by a component which is capable of being redistributed in a manner which is related to the degree of the influence,

as a change in light intensity wherein the illumination is provided by a laser which is scanned in a raster fashion over some or all of the spatial limitations being measured, the

~~scanning taking place at a rate substantially faster than the measurement process such that the illumination appears to the measurement process to be continuous in time and spatially uniform over the region being measured.~~

2. (Currently Amended) The method ~~A method~~ according to claim 1, wherein translocation is caused by an influence ~~the quantitative information which is indicative of the degree of the cellular response to the influence or the result of the influence on the subcellular component is extracted from the recorded variation according to a predetermined calibration based on responses or results, recorded in the same manner, to known degrees of a relevant specific influence.~~

3. (Currently Amended) The method ~~A method~~ according to claim 2 ~~claim 1~~, wherein the influence comprises contact between the mechanically intact or permeabilised living cells and a chemical substance and/or incubation of the mechanically intact or permeabilised living cells with a chemical substance.

4. (Currently Amended) The method ~~A method~~ according to claim 1, wherein the cells comprise a group of cells contained within a spatial limitation.

5. (Currently Amended) The method ~~A method~~ according to claim 1, wherein the cells comprises multiple groups of cells

contained within multiple spatial limitations.

6. (Currently Amended) The method ~~A method~~ according to claim 1, wherein the spatial limitations are spatial limitations arranged in one or more arrays on a common carrier.

7. (Currently Amended) The method ~~A method~~ according to claim 6, wherein the spatial limitations are wells in a plate of microtiter type.

8. (Currently Amended) The method ~~A method~~ according to claim 1, wherein the redistribution translocation results in quenching of luminescence fluorescence, the quenching being measure as a decrease in the intensity of the luminescence fluorescence.

9. (Currently Amended) The method ~~A method~~ according to claim 1, wherein the redistribution translocation results in energy transfer, the energy transfer being measure measured as a change in the intensity of the luminescence.

10. (Currently Amended) The method ~~A method~~ according to claim 1, wherein the intensity of the light being ~~recorded~~ is a function of the luminescence fluorescence lifetime, polarization, wavelength shift, or other property which is modulated as a result of ~~an~~ the underlying cellular response.

11. (Currently Amended) ~~The method~~ A method according to claim 1, wherein the light to be measured passes through a filter which selects the desired component of the light to be measured and rejects other components.

12. (Cancelled).

13. (Currently Amended) ~~The method~~ A method according to claim 1, wherein the ~~luminophore is fluorescence comes from a~~ luminescent polypeptide, ~~such as GFP.~~

14. (Currently Amended) ~~The method~~ A method according to ~~claim 13~~ claim 1, wherein the luminescent polypeptide ~~could be is~~ a ~~fluorescent polypeptide~~ GFP selected from the group consisting of ~~green fluorescent proteins having the F64L such as F64L-GFP, F64L-Y66H-GFP, F64L-S65T-GFP, and EGFP.~~

15. (Currently Amended) ~~The method~~ A method according to claim 1, wherein the cells are selected from the group consisting of ~~fungal cells, such as yeast cells, invertebrate cells including insect cells, and vertebrate cells, such as mammalian cells.~~

16. (Currently Amended) ~~The method~~ A method according to ~~claim 2~~ claim 15, wherein the mechanically intact or

permeabilised living cells are mammalian cells which, during the time period over which the influence is observed, are incubated at a temperature of 30°C or above, ~~preferably at a temperature of from 32°C to 39°C, more preferably at a temperature of from 32°C to 38°C, and most preferably at a temperature of about 37°C.~~

17. (Currently Amended) The method ~~A method~~ according to claim 1, used as a screening program.

18. (Currently Amended) The method ~~A method~~ according to claim 17, wherein the method is a screening program for the identification of a biologically active substance that directly or indirectly affects an intracellular signalling pathway and is potentially useful as a medicament, wherein the result of the individual measurement of each substance being screened which indicates its biological activity is based on measurement of the translocation ~~redistribution~~ upon activation of an intracellular signalling pathway.

19. (Currently Amended) The method ~~A method~~ according to claim 17, wherein the method is a screening program for the identification of a biologically toxic substance as defined herein that exerts its toxic effect by interfering with an intracellular signalling pathway, wherein the result of the individual measurement of each substance being screened which indicates its potential biologically toxic activity is based on

measurement of the translocation redistribution of said fluorescent probe in living cells and which undergoes a change in distribution upon activation of an intracellular signalling pathway.

20. (Previously Presented) A set of data relating to an influence on a cellular response in mechanically intact or permeabilised living cells, obtained by a method according to claim 1.

21. (New) The method according to claim 1, wherein the translocation is from cytoplasm to membrane or from membrane to cytoplasm.

22. (New) The method according to claim 1, wherein the translocation is from an aggregated form to a dispersed form or from a dispersed form to an aggregated form.

23. (New) The method according to claim 14, wherein the fluorescent polypeptide is a Green Fluorescent Protein (GFP).

24. (New) The method according to claim 23, wherein the GFP has a F64L mutation.

25. (New) The method according to claim 24, wherein the GFP is selected from the group consisting of F64L-GFP, F64L-Y66H-GFP,

F64L-S65T-GFP, and EGFP.

26. (New) the method according to claim 1, wherein said luminophore is a fluorophore.

27. (New) The method according to claim 15, wherein said fungal cells are yeast cells, said invertebrate cells are insect cells and said vertebrate cells are mammalian cells.

28. (New) The method according to claim 16, wherein said temperature is from 32°C to 39°C.

29. (New) The method according to claim 16, wherein said temperature is from 32°C to 38°C.

30. (New) The method according to claim 16, wherein said temperature is about 37°C.

31. (New) A method for detecting translocation of a component fused to a fluorophore in a cell in mechanically intact or permeabilised living cells, the method comprising:

providing said component fused to said fluorophore, wherein said fluorophore is encoded by and expressed from a nucleic acid sequence in said cell;

contacting said mechanically intact or permeabilised living cells with a chemical substance and/or incubating the

mechanically intact or permeabilised living cells with a chemical substance; and

detecting translocation of light emitted from said fluorophore, wherein said translocation is from cytoplasm to membrane, from membrane to cytoplasm, from an aggregated form to a dispersed form, or from a dispersed form to an aggregated form.